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Moulting and Morphogenetic Aberrations Induced by Diflubenzuron in *Spodoptera mauritia*

V JAGANNADH and V S K NAIR*

Department of Zoology, University of Calicut, Calicut 673 635

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Variously aged penultimate (fifth)/last (sixth) instar larvae and newly ecdysed pupae of *Spodoptera mauritia* were topically treated with different doses of diflubenzuron (DFB) and the effects on moulting and metamorphosis were studied. DFB treated insects showed different degrees of ecdysial failures and the formation of larval-pupal intermediates, defective pupae and adultoids. The significance of these findings is discussed.

Key Words : ***Spodoptera mauritia*, Diflubenzuron, Moulting, Metamorphosis. 12**

Introduction

Diflubenzuron (DFB) and several related benzoylphenyl ureas (BPUs) specifically interfere with the deposition of chitin in insects and thereby inhibit the moulting process. Biological activities and pesticidal properties of DFB and other BPUs have been extensively investigated and reviewed (Reynolds 1987, Darvas & Varjas 1990). BPUs possess three favourable characteristics which make them ideal choice for inclusion in integrated pest management programmes. The biggest advantage of BPUs is their proven selectively to insects. The other advantage is their low mammalian toxicity. Here we present results of laboratory investigation on the morphogenetic effects of DFB on larval development and metamorphosis of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae).

* Author for correspondence

Materials and Methods

Fifth instar (penultimate) larvae, sixth instar (last larval instar) larvae and pupae were taken from the laboratory colony of *S. mauritia* reared and maintained as described earlier (Nair 1981). The age of the larve was classed as day *n* where day O indicates the day that a larva ecdyses to a given instar.

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-diflurobenzoyl) urea], technical grade (97% purity) was a gift from M/s Duphar, B.V., Weesp, Holland. The compund was dissolved and diluted in acetone so as to obtain different concentrations ranging from 0.5 to 40 µg/5 µl of the solvent. The larvae and pupae of *S. mauritia* were topically treated with DFB along the dorsal midline utilizing a Hamilton microsyringe. Controls received an equivalent volume of acetone only.

Results

Effects of Treatments of Fifth Instar Larvae

Fifth instar larvae of various age groups (day 0, day 1 and day 2) were treated topically with three different concentrations of DFB, *viz.*, 0.5, 1 and 2 µg, which were not toxic as the percentage of larval mortality immediately after treatment was not significant (table 1). Most of the mortality occurred during ecdysis to the next stage. The larvae were unable to emerge from old larval exuviae. The larvae with partially slipped head capsule and body covering were also noticed (figure 1). Due to retention of old head capsule, the larvae were unable to feed normally. The newly formed cuticle seemed to be very thin producing lesions over larval body.

Effects of Treatments of Sixth Instar Larvae

Variously aged sixth instar larvae (day 0 - day 5) were treated topically with 0.5 µg, 1 µg or 2 µg DFB and the effects on larval-pupal transformation were carefully observed. All the three doses tested were effective in preventing larval-pupal development (table 2). Most of the larvae moulted into larval-pupal intermediates. The larval pupal intermediates showed pupal characteristics on the abdominal and thoracic region, whereas the head and legs were larval in appearance (figure 2). A secretion, apparently moulting fluid was seen entrapped between old larval and pupal cuticle. Defective pupae which were unable to cast off the larval exuviae were also noticed (figure 3). All these larval-pupal intermediates and defective pupae were not viable, they died within a day or two (table 2).

Effects of Treatments of Pupae

For this study day o pupae were treated topically with four different

Table 1 *Effects of treatments of DFB on variously aged fifth instar larvae of S. mauritia*

Age of the larvae	Dosage (µg)	*n*	% Mortality as fifth instar	%Surviving larvae which showed Moulting into fifth instar	sixth instar	Incomplete Moulting and death
Day 0	0.5	20	-	70	30	
		1	25	5	60	40
		2	21	9.5	0	100
Day 1	0.5	20	-	50	50	
		1	28	-	51	49
		2	25	-	13.33	86.66
Day 2	0.5	22	5	85.72	14.28	
		1	26	5	80.2	19.8
		2	28	10	81.81	18.18
Control	5µl	20	-	100	-	acetone each

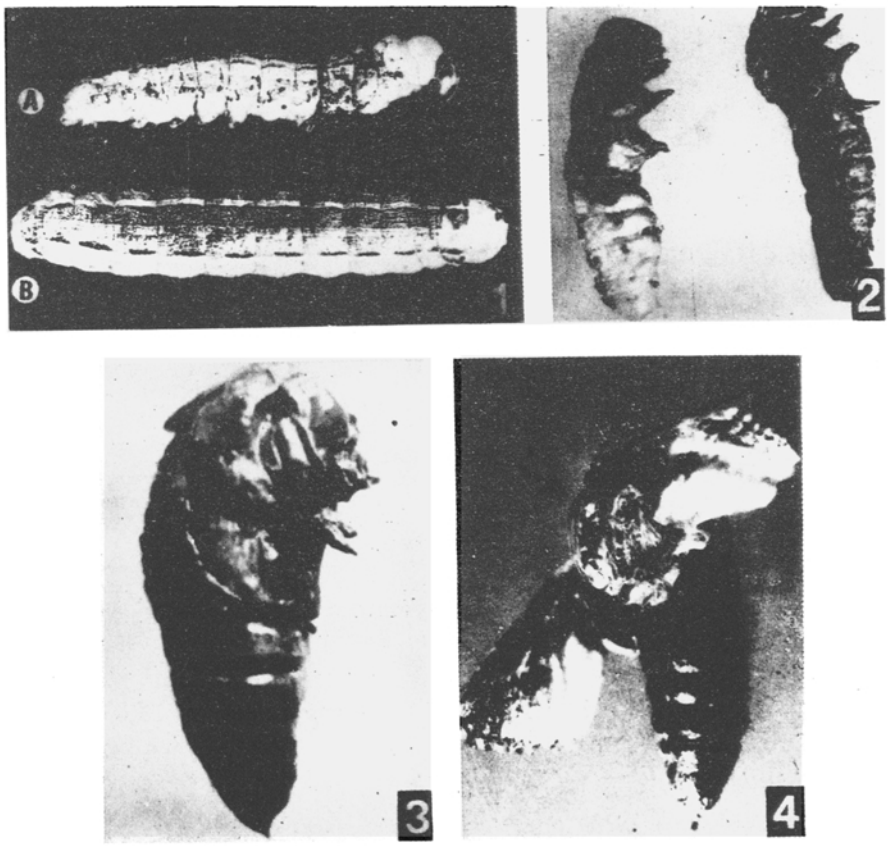

Figure 1 Partially moulted sixth instar larva (A) after treatments of DFB. Control sixth instar larva (B) *Figure 2* Larval-pupal intermediates formed after treatments of sixth instar larvae with DFB *Figure 3* Defective pupa formed after treatments of sixth instar larva with DFB *Figure 4* Adultod formed after treatment of day o pupa with DFB. [Note the retention of pupal cuticle and bifurcated proboscis (arrow)]

Table 2 *Effects of treatments of DFB on variously aged sixth instar larvae of S. mauritia*

Age of the larvae	Dosage (µg)	*n*	% Larval mortality	Number of larve transformed into : Larval-pupal intermediates		Normal pupa		
Day 0	0.5	5	20	17	3			
		1	25	12	20	2		
		2	24	20.83	20	-	5	
		22	45.45	12	-			
Day 1	0.5	25	-	23	2			
		1	25	8	22	1		
		2	22	18.18	18	-	5	
		30	23.33	23	-			
Day 2	0.5	25	-	11	14			
		1	25	-	20	5		
		2	20	-	16	4	5	30
		16.66	25	-				
Day 3	0.5	25	-	5	20	1	25	-
23	2							
		2	22	-	22	-		
		5	30	13.33	26	-		
Day 4	0.5	20	-	4	16			
		1	24	-	21	3	2	20
		-	18	2				
		5	30	30	21	-		
Day 5	0.5	20	-	18	2	1	25	10
17	2							
		2	20	-	20	-	5	20
		10	19	-				
Control 5µl	15	-	-	15	acetone each			

concentrations of DFB viz., 5µg, 10µg, 20µg and 40µg. Pupae treated with 5µg DFB emerged normally within 7-8 days as in the case of controls. As the dosage of DFB increased, the number of pupae showing normal eclosion decreased (table 3). Administration of 40µg resulted in prevention of eclosion in 60% of treated pupae. The unemerged pupae were found to contain adultoids with partially folded wings and bifurcated proboscis (figure 4). A few of the adultoids which emerged were seen to retain the old pupal cuticle. Adultoids were to unable feed and did not mate.

Table 3 *Effects of treatments of DFB on day o pupae of S. mauritia*

Dosage (µg)	*n*	% Mortality	Number of pupae transformed into : Adultoids	Adults
5	20	10	-	18
10	25	16	2	19
20	30	16.66	12	13
40	35	17.14	21	10
Control	20	-	-	20 5µl acetone each

Discussion

This study clearly demonstrates that the most common symptom associated with DFB treatments in *S. mauritia* is the disruption of ecdysis. In the present study DFB is found to be very effective in inhibiting moulting of treated larvae/ pupae and induce the formation of cuticular lesions. DFB is known to cause such cuticular lesions and moulting aberrations because of its mode of action. DFB and related BPUs act primarily by disrupting the formation of insect cuticle. The BPUs selectively inhibit the final stage of *in vivo* chitin synthesis especially the formation of chitin synthetase or glucosyltransferases (Reynolds 1987). In DFB treated insects the newly formed cuticle consisting of epicuticular and endocuticular tissue is not properly attached to the epidermis. Moreover, the deficiency of chitin deposition further weakens the rigidity of newly formed cuticle. Hence the newly formed cuticle is a very delicate one which cannot resist muscular traction and increased turgor during moulting. This explains why the affected larvae succeed in only partly or not at all in casting off their exuviae.

In *S. mauritia,* treatments of DFB seriously affect the larval development and metamorphosis. The surviving larvae/pupae ecdyse into defective pupae/adults. Formation of larval-pupal/ pupal-adult mosaics after DFB treatments have been observed in several holometabolous insects (Reed & Bass 1980, Gordon et al. 1989, Gujar & Mehrotra 1989, Mayuravalli et al. 1989).

The disruption of moulting and the formation of larval-pupal/pupal-adult intermediates have often been interpreted as due to the interaction of DFB with the insects morphogenetic hormones. One earlier proposal is that BPUs cause an inhibition of ecdysteroid metabolizing enzymes resulting in a build up of moulting hormone levels (Kobayashi et al. 1989). However several studies have failed to confirm this model of BPU action. For example in the last instar larvae of Oncopeltus, the ecdysteroid peak was delayed by DFB (Redfern et al. 1981) and in Tenebrio pupae it was completely prevented (Soltani et al. 1989).

From an endocrinologist's point of view the formation of larval-pupal intermediates after DFB treatments is a symptom of excess juvenile hormone (JH) titre. This led several investigators to

suggest that the formation of larval-pupal and nymphal-adult mosaics is due to hyper JH titre (Mariy et al. 1981, Lim & Lee 1982, Gujar & Mehrotra 1986, Rao & Mehrotra 1986). Further rectal prolapse which is a common symptom of excess JH have been reported in DFB treated Pseudoplusia includens (Reed & Bass 1980) and *Spodoptera litura* (Negishi et al. 1986). Earlier studies conducted in this laboratory have shown that treatment of variously aged sixth instar larvae of *S. maurita* with a JH analogue induced age dependent effects with day 3 larvae moulting into superlarvae (Santha & Nair 1987). As seen in the present study treatment of sixth instar larvae of *S. mauritia* with DFB causes no such effect. These observations strongly suggest that at least in the case of *S. mauritia* DFB does not induce a hyper JH effect. From such contradictory reports, relationship between DFB and morphogenetic hormones appear unclear.

Alternatively DFB treatments have also been shown to prevent the proliferation of undifferentiated primordial cells which form the imaginal epidermis thereby blocking the subsequent cycle of cuticle deposition (Meola & Mayer 1980). Moreover, DFB inhibits DNA synthesis in the imaginal epidermal histoblasts which develop into adult epidermal cells (DeLoach et al. 1981). These studies clearly show that many of the morphogenetic effects of DFB could be explained by the effects of the compound on chitin synthesis and imaginal precursor cells. It is possible that the observed changes in hormone titre is a consequence rather than a cause for the morphogenetic changes.

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